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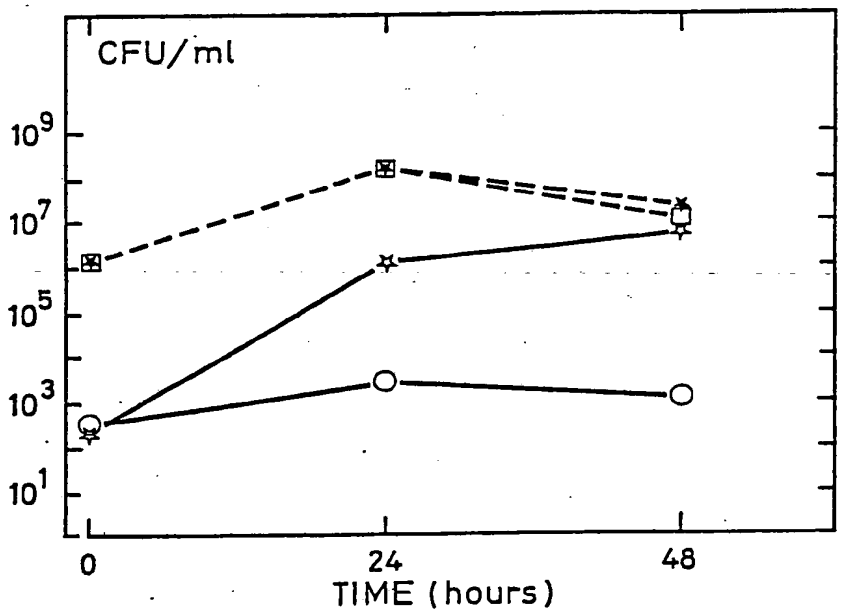

(57) Abstract

Soluble gelatin capsules for controlling vulvo-vaginal infections contain a stable, dried, variable concentrate, such as a freeze-dried concentrate, of lactid acid bacteria, especially *Lactobacillus acidophilus*, dispersed in a pharmaceutically active fluid carrier which has a viscosity of at least 2000 cps, preferably 3000 cps, and in particular 4000 cps. The count of bacteria per capsule is at least $1x10^6$, preferably at least $1x10^7$. The water activity (a_w) of the bacterial concentrate and the fluid carrier is in the range of 0.00-0.2. The fluid carrier is favourably a non-hygroscopic oil containing a viscosity-increasing agent such as starch and/or vaseline. The resulting capsules have been found to have an inhibitory activity against *Candida albicans in vitro* and *in vivo*.

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VAGINAL CAPSULES

The present invention relates to a method and a capsule for controlling vulvo-vaginal infections.

It has been known for a long time to use antibiotics in the treatment of vulvo-vaginal infections, but antibiotic treatment has proved to be disadvantageous as antibiotics tend to kill the desired microflora in the vagina as well, whereby the natural healthy microbial balance of the vagina is disturbed. This in turn often results in the condition becoming re-occurring as the pathogenic microorganisms often re-invade the vaginal environment without other microorganisms to check their growth.

A more preferred way of controlling vulvo-vaginal infections is to employ lactic acid bacteria so as to simulate the normal vaginal environment. There is some disagreement as to the causes of vulvo-vaginal infections. It has been thought that infections occur when the pH of the vagina is too high such as at menstruation, or when the hormonal balance is disturbed because of pregnancy or when taking oral contraceptives, thus promoting the growth of potentially pathogenic microorganisms such as *Candida albicans*, *Trichomonas vaginalis*, *Staphylococcus aureus*, *Gardnerella*, β-*Streptococci* and various anaerobic microorganisms which, conversely, were not assumed to thrive under the normal, slightly acidic conditions in the vagina. Thus it was believed that, by introducing lactic acid bacteria in the vagina, the environment would attain its normal acidity so that the propagation of the pathogenic microorganisms would once more be inhibited.

However, more recent studies (cf. *Annals of Internal Medicine* vol. 96 (Part 2), 1982, pp. 931-34, *J. Clin. Microbiol.*, May 1980, pp. 479-84, *Br. J. Vener. Dis.* vol. 56, 1980, pp. 107-10) indicate that it is not the acidity of the vagina as such which prevents the excessiv growth of undesirable microorganisms as both *Candida* and *Trichomonas* grow under acidic conditions, but rather the ecological balance normally prevailing in the vagina, or rather on the vaginal mucosa, and that infections are more likely to occur when, for some reason,

the population of the various *Lactobacillus* species has become diminished. The studies cited agree that there is a correlation between the abundance of *Lactobacillus* colonies and vaginal pH, in that the pH decreases with the number of *Lactobacilli* present, but that it is the actual presence of large numbers of *Lactobacilli* which prevents vulvo-vaginal infections.

In the known use of lactic acid bacteria, either a fermented milk product containing *Lactobacilli* is introduced manually, or a slurry of dried *Lactobacillus acidophilus* (e.g. the product known as Floranorm, marketed by Danapharm) is introduced by means of a suitable applicator such as a disposable syringe.

It has also been suggested to introduce lactic acid bacteria incorporated in a capsule, *vide* US Patent No. 3,639,566 which discloses a hard gelatin capsule which contains estriol and living Doederlein bacteria (the type of *Lactobacilli* naturally occurring in the vagina) adsorbed to starch. This product has a moisture content of about 40%.

The non-encapsuled products, however, suffer from the disadvantage that their application is uneven, i.e. the distribution of the lactic acid bacteria in the vagina is not homogeneous. The application method may also be found to be inconvenient. Also, as the products are rather fluid, they tend to run out of the vagina so that they do not have the desired effect. A disadvantage of the product known from US Patent No. 3.639.566 is that the bacteria incorporated in the capsules are likely to have a limited stability, due to the content of moisture absorbed by the starch incorporated in the capsule so that the capsules may only be stored for limited periods of time. Furthermore, when applied in the vagina, the capsule will take up moisture for which reason the capsule content tends to get lumpy; this may result in an uneven distribution over the vaginal area and probably a reduced effect.

The present invention provides capsules which are superior with respect to efficient introduction and distribution of an effective amount of lactic acid bacteria into the vagina, and correspondingly

provides an efficient method for controlling vulvo-vaginal infections. The capsules of the invention are improved over the known art by containing stabilized cells of lactic acid bacteria, which means that they may be stored at room temperature for several months while maintaining a high percentage of viability.

Thus, one aspect of the invention relates to a soluble capsule for controlling vulvo-vaginal infections which contains a stable, dried, viable concentrate of lactic acid bacteria dispersed in a pharmaceutically acceptable fluid carrier.

In the present context, the term "dried" indicates that the concentrate has a water activity (a_w) of not more than 0.2, calculated according to the formula described by e.g. D. Demeyer, *Fleischwirtschaft* 59(7), 1979, p. 940.

The capsule is preferably a gelatin capsule, in particular a soft gelatin capsule. The capsule may, however, also be a hard, but soluble gelatin capsule. The capsule may be of any suitable shape such as a spherical or oblong shape, but for easy application it is preferred that the capsule be oval or egg-shaped. The volume of the capsule is in the range of about 0.2-5.0, preferably about 1.2 ml. The capsule volume is in fact rather critical as the physical properties of the paste (*vide* below) set limits to the amount of paste material and thus the amount of bacteria per capsule which may adhere or be adsorbed to the vaginal mucosa without causing any significant discharge problems. The capsules preferred for use according to the invention are those produced substantially according to the disclosure of US Patents Nos. 1,970,396, 2,152,101, 2,234,479 and 2,288,327.

It is well known to prepare concentrates of lactic acid bacteria. Methods for preparing such concentrates are described, e.g., in US Patent No. 4,115,199, utilizing a polyphosphate salt such as sodium hexametaphosphate or sodium tripolyphosphate as an additive to the culture medium prior to concentration by centrifugation. The wet bacterial concentrate is then dried, preferably freeze-dried, under stabilizing conditions in the presence of stabilizing additives as described in US Patent No. 3,897,307.

By using a freeze-dried concentrate, it becomes possible to obtain a high count of stable, viable bacteria in a capsule for intravaginal administration. According to the invention, the count of viable lactic acid bacteria is at least 1×10^6, preferably at least 1×10^7, more preferably at least 1×10^8 and most preferably in the range between about 1×10^9 and 1×10^{11}, such as about 1×10^9 and 1×10^{10} p r capsule.

The dried, stabilized bacterial concentrate, when incorporated in the capsules of the invention, is not totally moisture-free, but contains some internal moisture. In order to obtain a stable, dried concentrate with such a high cell count of viable bacteria, it is, however, desirable that the water activity of the concentrate is in the range of 0.00-0.2, preferably in the range of 0.00-0.1. A suitable instrument for determining water activity is available from Novasima AG, Zürich, Switzerland.

In order to prevent an excessive uptake of water and oxidation of the concentrate, resulting in a substantial loss of viability, which is likely to occur if the dried concentrate was to be incorporated in the gelatin capsules as such, the concentrate is mixed with a fluid carrier. According to the invention, it has been found that particular types of fluid carrier are extremely well suited both for an even distribution in the vagina and for maintaining a high stability (i.e. long-term viability) of the concentrate.

The carrier is thus primarily so selected that it will protect the freeze-dried bacterial culture from physical and/or chemical reactions which have a negative effect on cell stability (expressed as a loss of viability). It has been found that the most important single factor for maintaining the stability of the concentrate is the low water activity of the culture/carrier mixture for the reasons stated above. The carrier should also have a water activity in the range of 0.00-0.2, preferably 0.00-0.1. The carrier is preferably a non-hygroscopic carrier to substantially prevent water uptake through the capsule wall which would decrease bacterial stability.

The fluid carrier should also be one which secures a homogeneous distribution of the bacteria in the vagina and an optimal contact with the vaginal mucosa upon the release of the carrier from the partly dissolved capsule but which, on the other hand, does not cause the carrier material to run from the vagina immediately after application. From a manufacturing point of view, though, it is normally advantageous that the carrier is relatively low in viscosity during processing conditions and during the filling of the capsules. Therefore, it is desirable that a carrier containing a bacterial concentrate has a certain thixotropicity, i.e. increase in viscosity, once it has been filled into the capsules so as to prevent sedimentation of the bacteria in the carrier and so as to prevent excessive movement of the carrier in the capsule in order to cause as little of the bacterial concentrate as possible to come into contact with the relatively wet capsule wall during the manufacturing process. This relative immobility minimizes the amount of moisture which might possibly be taken up by the dry bacterial concentrate which might in turn cause a decrease of bacterial stability. Also, when such capsules are stored at normal storage temperatures, it is believed to be an advantage that the viscosity of the carrier within the capsule is sufficiently high to ensure that there is little movement of the carrier during handling or transportation of the capsules, thereby avoiding any movement of the dried concentrate which might increase the exposure of the concentrate to the inner capsule wall and thus increase the possibility that the culture might receive moisture from the surroundings. This emphasis on a certain viscosity of the fluid carrier distinguishes the capsule of the invention from capsules intended for oral administration, which contain lactic acid bacteria in a fluid carrier (e.g. known under the trade name Lactoflora™, marketed by Camette ApS, Esbjerg, Denmark, as the fluid carrier in the known capsules does not have a sufficient viscosity to prevent sedimentation of the bacteria and possible uptake of moisture so that cell stability is likely to be impaired.

Consequently, the capsule of the invention should contain a fluid carrier with a viscosity of at least 2000 cps at a temperature of 20°C, as this viscosity is sufficient to secure a satisfactory cell stability, while not being too high to prevent a "melting down" in the vagina to ob-

tain a sufficiently low viscosity at the vaginal temperature for the above-mentioned homogeneous distribution to take place. It has been found that when the carrier has a viscosity of at least 3000 cps, in particular at least 4000 cps at 20°C, it will still have a behaviour with respect to consistency which is very well suited for effective distribution of the bacterial culture under the conditions prevailing in the vagina. A capsule containing such a carrier with a concentrate of lactic acid bacteria may suitably be administered when the patient goes to bed. A few minutes after administration, the capsule will disintegrate to such an extent that it releases its carrier content which is then distributed in the vagina, causing an even distribution of the concentrate of viable lactic acid bacteria which adhere to the vaginal wall and, in the conditions prevailing in the vagina, such as temperature and moisture, will become biologically active and multiply. When the patient stands, any remainder of the carrier will tend to leave the vagina, but will not give rise to noteworthy discomfort.

In accordance with the present invention, the fluid carrier is a substantially anhydrous paste preferably comprising a substantially non-hygroscopic oil which is either inherently of a suitable viscosity, e.g. comprises a mixture of oil and fat, such as cocoa butter, or which may include a particulate or dissolved or polymeric viscosity-increasing agent to obtain a suitable viscosity.

The viscosity-increasing agent may be selected from solid or semi-solid hydrocarbons capable of forming a homogeneous system with the oil, such as vaseline or polyethylene, particulate inorganic substances such as fumed silica, talc, zeolite or bentonite, and carbohydrates or carbohydrate derivatives, preferably high molecular weight carbohydrates such as starch and starch derivatives. It is, however, believed to be necessary that the viscosity-increasing agents employed should have as low a water activity as possible; preferably the a_w does not exceed 0.1. This means that the viscosity-increasing agent should have a certain, but limited hygroscopicity. Thus, sugars such as dextrose or maltodextrine have been attempted as viscosity-increasing agents, but have proved unsuitable due to their poor adsorption thermes, i.e. the amount of water bound to the adsorbent per defined increase in water activity.

According to the invention, it has been found that a combination of an oil and a carbohydrate derivative such as a starch, in particular corn starch, is especially advantageous for maintaining a high stability of the dried lactic acid bacteria concentrate dispersed therein.

Furthermore, an oil and corn starch mixture is the preferred carrier because it has been found that there is a synergism between a substantially anhydrous oil and corn starch with respect to preserving the viability of lactic acid bacteria. Although the present invention is not to be limited to any theory, it is believed that the synergism is a combined effect of 1) the fact that the corn starch (which is preferably in a freeze-dried or dehydrated form prior to its incorporation in the oil so as to have a water activity of almost 0:00) has a balanced water activity which tends to attract water from the oil in the final system, thus competing with the dried bacterial culture (which is very hygroscopic in the freeze-dried state) which will also have a tendency to absorb any small amount of free water present in the oil, and 2) the starch such as corn starch in particular may have an inherent stabilizing effect on lactic acid bacteria. However, in order to avoid any substantial dessication of the capsules which may be caused by the incorporation of freeze-dried or dehydrated starch, the starch may advantageously be admixed with vaseline in an amount of about 10-50% by weight of the starch. Thus, a combination of a substantially anhydrous oil and the starch such as corn starch or, especially, the starch/vaseline mixture, e.g. a weight ratio in the range from about 2:5 to about 5:2, preferably about 1:1, has been found to be a most suitable carrier for efficiently intravaginally administering lactic acid bacteria.

As examples of useful oils may be mentioned mineral or vegetable oils such as paraffin or sunflower oil.

The lactic acid bacteria concentrate incorporated in the capsules according to the invention may be comprised of any type of bacteria which produce lactic acid, such as bacteria belonging to the genus *Streptococcus* or *Lactobacillus*. The species of *Lactobacillus* employed according to the invention are principally *L. acidophilus*, *L. bulgari-*

cus, *L. lactis*, *L. helveticus*, *L. bifidus*, *L. casei*, *L. plantarum*, *L. delbrueckii*, *L. thermophilus* or *L. fermentum*. Preferred among *Streptococcus* species are *S. lactis*, *S. cremoris*, *S. diacetylactis*, *S. thermophilus* or *S. faecium*. The lactic acid bacteria may also be incorporated in the form of a mixture of two or more of these species. Lactic acid bacteria of the species *L. acidophilus* have proved particularly advantageous. The strain of *L. acidophilus* which has proved to be particularly advantageous has been deposited in the Northern Regional Research Center, Peoria, USA under the accession number NRRL No. B-15260 and is publicly available.

The conditions treated by administering the capsule according to the invention are vulvo-vaginal infections caused by, i.a., microorganisms such as *Candida albicans*, *Trichomonas vaginalis* and *Staphylococcus aureus* as well as various anaerobic microorganisms. The capsule of the invention has been found to be particularly advantageous in the treatment of recurrent vulvo-vaginal infections which has hitherto been difficult to cure with conventional preparations such as antibiotics. As mentioned above, a particularly advantageous species of lactic acid bacteria is *L. acidophilus*, as described in Example 5. The superior qualities of *L. acidophilus* are most likely due to the fact that this species not only produces lactic acid (a decrease of pH is often not enough to control or reduce the growth of pathogenic microorganisms), but has also been found to produce one or more antimicrobial metabolites described as acidophilin (*vide* e.g. Shahani, K.M. et al: "Natural Antibiotic Activity of *Lactobacillus acidophilus* and *bulgaricus*. 2. Isolation of Acidophilin from *L. acidophilus*", *Cult. Dairy Prod. J.* 12, 1977, p. 8.), acidolin (*vide* e.g. "*Lactobacillus acidophilus* II. Antimicrobial agents, *Cult. Dairy Prod. J.* 10, 1975, p. 18), and lactosidin (*vide* e.g. "Antibacterial activity associated with *Lactobacillus acidophilus*", *J. Bacteriol.* 78, 1959, p. 477). These antimicrobial agents have been shown to possess an inhibitory effect on a variety of microorganisms.

Less severe cases of vulvo-vaginal infections may suitably be treated by administering 1-2 capsules a day for 3-6 days, or a similar dosage may be administered prophylactically for a few days after each men-

struation. A suitable dosage in more severe cases may be 1 capsule a day until the first menstrual period, and for a 7-day period after each menstruation. The capsule may be inserted with the fingers or by means of a suitable applicator.

The capsules may be produced by homogeneously dispersing a dried, viable, stable culture of lactic acid bacteria in a substantially anhydrous fluid carrier, filling the resulting dispersion into soft gelatin capsules and drying the capsules after sealing.

More specifically, in order to make the mixing of the ingredients relatively easy, especially if the paste is to include a viscosity-increasing agent, it is preferred to add the culture to the paste ingredient which has the lowest viscosity followed by adding the viscosity-increasing agent in the form of other dry matter or optionally a more highly viscous ingredient. This procedure saves time which is vital with respect to the amount of moisture taken up by the culture from the air. In order to avoid moisture uptake, an inert and dry gas may be exposed to the mixing surface.

The admixture of the culture and optionally other dry matter and the oil may, for instance, be performed by means of a slowly operating mixer. In this way, the uptake of air in the mixture is minimized, thus minimizing the risk of uptake of moisture and oxygen which are detrimental to bacterial stability. In order to further reduce the moisture content of the final product, the ingredients are incorporated in the oil in a dry state. Thus, the freeze-dried bacterial concentrate has a water activity not exceeding 0.2 and preferably a far lower water activity, and when the viscosity-increasing agent employed is a starch such as corn starch, it is preferably subjected to freeze-drying prior to use, substantially to a water activity of 0.00.

However, a finely dispersed and homogeneous product is not obtained by merely mixing the ingredients as described above, and is provided e.g. by dispersing the culture by means of a roller mill operating at the pressure and friction by which a fine division of the bacterial

culture is secured without, on the other hand, heating the paste or killing the bacteria. The space between the rollers will normally be between 150 and 300 µm. The resulting particle size will be about 100 µm. After this grinding process, the paste is gently stirred in order to impart homogeneity to the paste which is important to obtain in order to make it possible to dose accurate amounts of bacteria into each capsule.

During the mixing and grinding processes, admixture of a certain amount of air in the paste is unavoidable. As stated above, this may have a detrimental effect on bacterial stability as well as influence the accuracy with which the capsules are filled. Production of the paste should therefore also include removal of air from the paste which is performed in a vacuum chamber at a pressure of 12 mm Hg in which the paste is atomized by means of a fast-rotating, horizontal plate. During the entire process, the paste is subjected to a total temperature increase of 3-5°C relative to room temperature.

The paste is then incorporated in gelatin capsules, preferably soft gelatin capsules, by a process described in US Patents Nos. 1,970,396, 2,152,101, 2,234,479 and 2,288,327. After shaping, filling and sealing, the capsules may be washed with an agent which prevents intercapsular adhesion, such as perchloroethylene, to which a lubricant such as lecithin has optionally been added whereby intercapsular adhesion and deformation of the capsules during the subsequent drying process are avoided. The drying itself may be performed in a two-stage process. Firstly, the still wet capsules may be subjected to a strong air-flow, normally with ordinary atmospheric air with a relative humidity of about 20-60%. When the capsules have become sufficiently hard, the drying is continued on trays with ventilation for up to several days until the desired hardness and loss of humidity have been obtained. For improved storage, the final product is preferably stored at a temperature below 20°C, such as at a refrigeration temperature of about 3-5°C.

The invention is further illustrated by the following examples.

EXAMPLE 1

Preparation of the Bacterial Culture

A dry, stable, viable concentrate of *L. acidophilus* was prepared according to the procedures described in US Patents Nos. 4,115,199 and 3,897,307. The strain of *L. acidophilus* used is deposited with the Northern Regional Research Center and is publicly available under the accession number NRRL No. B-15260. The bacterial concentrate was dried by freeze-drying after adjusting the pH to 6.0-6.2, and the addition of 16 g L-ascorbic acid, 10 g inositol and 10 g monosodium glutamate per 100 g of the dry concentrate. The resulting stabilized, dry concentrate contains about 1.00×10^{11} CFU (colony-forming units) per gramme.

EXAMPLE 2

Production of Paste

The compositions stated below were prepared according to the method described above with the exception of composition C.

The amount of paste material varies according to the desired number of cells in the finished capsules and thus the amount of bacterial concentrate incorporated, in order to obtain the desired viscosity.

Composition A:

Paraffin oil of low viscosity[1]	5.5 kg
Freeze-dried corn starch CPC 3401	4.5 kg
Freeze-dried *L. acidophilus* culture (1.05×10^{11} CFU/g)	1.0 kg

[1] as described in *Deutsches Arzneibuch*, vol. 7/8

This composition is presently preferred.

Composition B:

Paraffin oil of low viscosity[1]	7.0 kg
yellow vaseline[2]	6.0 kg
L. acidophilus culture (1.05×10^{11} CFU/g)	1.2 kg

[1] as described in *Deutsches Arzneibuch*, vol. 7/8
[2] as described in *Deutsches Arzneibuch*, vol. 7

Composition C:

Plastibase[1]	9.4 kg
"Alkathene" 23 powder[2] 2.5%	
Liquid paraffin[3] 97.5%	
L. acidophilus culture (1.05×10^{11} CFU/g)	1.0 kg

[1] prepared according to *Farmaceutisk Tidende*, No. 10, vol. 67, 1957, pp. 113-15
[2] a polyethylene powder (023.030) marketed by ICI

EXAMPLE 3

Comparison and Stability Tests

As described above, it is a requirement that the carrier for the bacterial culture has as low an uptake of water as possible, and preferably no uptake of water at all.

In order to determine the protective properties of various carrier materials, especially with respect to uptake of water from the surroundings, hygroscopicity analyses were made of the following materials and mixtures of materials as shown in Table 1. The materials

were spread on petri dishes with a diameter of 9 cm, and a thickness of the material of 7-8 mm, the materials having a substantially smooth surface.

The materials analyzed were:

Paraffin oil	Paraffinum liquidum tenui as described in *Deutsches Arzneibuch*, vol. 8, purchased from Mecobenzon.
Sunflower oil	Commercial edible oil purchased from Irma.
Corn starch	Globe® 03401 purchased from CPC, freeze-dried to an A_w of 0.00.
Culture	Freeze-dried concentrate of *L. acidophilus* NRRL No. B-15260 (1×10^{11} CFU/g), produced by Christian Hansen's Laboratories.
"Plastibase"	As described in *Farmaceutisk Tidende* No. 10, vol. 67, 1957, pp. 113-15, 2.5% polyethylene in liquid paraffin.
Vaseline	Vaselinum Ph. Nord. 63 purchased from Mecobenzon.

Table 1

Analysis of Hygroscopicity (% weight increase)[1]

Composition (% by weight)	2 weeks	3 weeks
Paraffin oil	0.1	-
Paraffin oil + culture (10%)	6.9	17.0[2]
Paraffin oil + corn starch (1:1) + culture (10%)	6.8	10.0
Sunflower oil	0.2	0.3
Sunflower oil + culture (10%)	6.3	9.7
Sunflower oil + corn starch (2:1) + culture (10%)	7.6	10.4

Sunflower oil + corn starch (1:1) + culture (10%)	14.9	18.4
Vaseline	0.0	0.0
"Plastibase" (2 1/2%) + culture (10%)	2.9	5.4
"Plastibase" (2 1/2%)	0.01	0.08
Corn starch	20.4	-
Culture (100%)	115.6	149.7

[1] defined and analysed as water uptake: % weight increase during 2-3 weeks by standing at 100% RH and 20°C (± 2°C).

[2] complete sedimentation of the culture from the oil.

It appears from the results that the water uptake of the oils, the vaseline and polyethylene is minimal, whereas the culture itself is extremely hygroscopic. When the culture is mixed with one or more of the carriers, the suitability of the selected carriers with respect to protection against water uptake may be seen. Addition of a viscosity-increasing agent such as corn starch which in comparison to the culture is only slightly hygroscopic does not appear to change the water uptake properties of the total mixture to any significant extent. However, the water uptake in the sunflower oil mixture is directly proportional to the amount of corn starch added. A mixture such as "Plastibase" is demonstrated to afford the best protection of the culture against water uptake.

With respect to protection against sedimentation, i.e. the viscosity of the paste, the following paste compositions were made:

1)	Vaseline	100 g	5)	Sunflower oil	100 g
	Paraffin oil	100 g		Culture	10 g
	Culture	20 g			
2)	Paraffin oil	100 g	6)	Sunflower oil	100 g
	Culture	10 g		Corn starch	50 g
				Culture	10 g

3)	"Plastibase" 2 1/2%	200 g	7)	Sunflower oil	50 g
	Culture	10 g		Corn starch	50 g
				Culture	10 g
4)	Paraffin oil	50 g	8)[1]	Corn starch	100 g
	Corn starch	50 g		Culture	10 g
	Culture	10 g			

[1] not prepared in a roller mill - the ingredients were mixed in a plastic bag

An unsatisfactory sedimentation of the culture on standing, i.e. too low a viscosity of the mixture, was only observed in mixtures 2) and 5).

Stability tests

1. The stability of the bacterial cultures when incorporated in various types of pastes or other carriers as described above was tested by means of an accelerated storage test (AST) in which 10 samples of each mixture of culture and carrier were subjected to increasing temperatures (from 30-75°C) in a water bath for 3 days, one sample of each mixture being removed from the water bath every 8th hour and analysed. The results are shown in Figs. 1-3.

In Figs. 1-3, the "white" star indicates the *L. acidophilus* culture alone, the square with the filled-in circle indicates oil and viscosity-increasing agent (paraffin oil and corn starch in Fig. 1, sunflower oil and corn starch in Fig. 2, paraffin oil and vaseline in Fig. 3), the square indicates the viscosity-increasing agent alone (corn starch in Figs. 1 and 2, vaseline in Fig. 3), the filled-in circle indicates the oil (paraffin oil in Figs. 1 and 3), and the filled-in star indicates "Plastibase" (as defined above); "log CFU" indicates the logarithm of colony-forming units per gramme of carrier.

It should be noted that the courses of the curves cannot be directly interpreted as stability measurements (percentage of loss of viability)

in a conventional storage test. However, the curves may be compared among themselves, especially with respect to the slope of the first, straight line and "breakpoint" (45°), i.e. the temperature at which the curve changes its course (usually at 59-60°C for cultures with a satisfactory stability; indicated by the dotted line in Figs. 1-3).

It appears from the Figures that the best results in terms of a slope approaching zero and a breakpoint at a temperature of about 59°C is obtained with the mixtures of oils (paraffin and sunflower oil) and corn starch even though each of the component parts of the mixtures shows a steeper course of the curves with breakpoints before or at 59°C. It further appears that the course of the curves is improved in comparison with the culture itself. Thus, in these mixtures, an improved protection has been obtained for the cultures, whereby the bacteria obtained a very high stability.

II. Capsules containing freeze-dried *L. acidophilus* bacteria dispersed in a mixture of sunflower oil and corn starch were also subjected to a conventional storage stability test in which the capsules were stored at 5°C and 20°C, respectively, and tested for their content of colony-forming units (CFU) after 4, 8, 16 and 32 weeks at the respective temperatures. The initial count of CFU's was 150 x 10^8/2 capsules. The results are shown in Table 2.

Table 2

	4 weeks %Survival	8 weeks %Survival	16 weeks %Survival	32 weeks %Survival
5°C	91	92	75	43
20°C	87	67	49	25

It appears from the table that the capsules have a half life of about 4 months at 20°C at which time the capsules still contain an effective amount of bacteria. Due to the improved storability at 5°C, it is, however, preferred that the capsules be stored at about 5°C or less.

EXAMPLE 4

Encapsulation and Drying

Soft gelatin capsules were prepared according to US Patents Nos. 1,970,396, 2,152,101, 2,234,479 and 2,288,327. The capsule material may vary in composition within certain limits. The composition preferred for the present purpose is shown in Table 3.

Table 3

Composition of Soft Gelatin Capsules

Gelatin[1]	189.040 - 221.916 mg
Glycerol[2]	62.411 - 73.265 mg
Anidrisorb 85/70	48.609 - 57.063 mg
Dyes[3]	
E 171/77891	3.288 - 3.860 mg
E 172/77492	0.252 - 0.296 mg
Total weight of capsule material per capsule	330 mg ± 8%

[1] according to USP

[2] 85% Ph. Eur.

[3] white, opaque, EWG Nos. and Col. Ind. 1956 Nos. listed

After shaping the capsules and filling them with the dispersion of bacterial culture incorporated in the paste and sealing, the capsules were washed in perchloroethylene to which had been added a limited amount of lecithin in order to avoid intercapsular adhesion and deformation of the capsules during drying.

The capsules were dried for 2 hours in a rotary drier with 6-8 compartments by a strong air-flow with ordinary atmospheric air with a relative humidity of about 20%. In this manner, the capsules were dried to an extent corresponding to a loss of weight of about 40-45%. The capsules had then become sufficiently hard for continued drying

to be performed on trays in a closed, ventilated cupboard at about 20°C (relative humidity 20%) for up to 4-6 days, until a suitable hardness and dessication had been obtained.

EXAMPLE 5

Inhibition of Candida albicans in vitro

To demonstrate the inhibitory effect of *L. acidophilus* on the growth of the yeast *Candida albicans* which is one of the most common causes of vulvo-vaginal infections in women, the following *in vitro* experiment was made.

A liquid growth medium (MRS-Oxoid CM 359 - 5.2%) was inoculated with the following lactic acid bacteria:

Lactobacillus acidophilus	NRRL No. B-15260
Lactobacillus bulgaricus[1]	CH-1
Streptococcus thermophilus[1]	CH-1

[1] Available from Chr. Hansen's Laboratories.

Each of the strains was inoculated in growth flasks with 100 ml MRS-broth to a cell count of 1-5 × 10^6 CFU/ml. To some of these flasks as well as to some flasks which had not previously been inoculated were simultaneously added one of two cultures of *Candida albicans* which were freshly grown from women with yeast infections (vulvo-vaginal infections). Furthermore, MRS-broth was inoculated with *L. acidophilus* in an amount of 5 × 10^6 CFU/ml and incubated at 37°C for 24 hours after which the living cells were centrifugated off and the supernatant were finally filtered under sterile conditions. The final pH was 4.18. This broth was inoculated with one of the *Candida* cultures. All flasks were incubated at 37°C, and the broths were plated for the specific microorganism after 0 hours, 24 hours and 48 hours.

The results are shown in Figs. 4-7.

In Fig. 4 a and b, the dotted lines represent the growth of *L. acidophilus* (expressed as cell count), the filled-in star indicating the growth of *L. acidophilus* when grown alone, and the square indicating the growth of *L. acidophilus* when grown together with *C. albicans* (two different strains in 4 a and 4 b). It appears that the growth of *L. acidophilus* is not inhibited by the presence of *Candida*. The full lines represent the growth of *C. albicans*, the "white" star indicating the growth of *Candida* when grown alone, and the circle indicating the growth of *Candida* when grown together with *L. acidophilus*. It appears that both *Candida* cultures are significantly inhibited (approx. 4 $\log_{10}$ units) when inoculated together with the *L. acidophilus* strain used.

Figs. 5 and 6 are analogous with Fig. 4 with the exception that Fig. 5 shows the use of *L. bulgaricus* and Fig. 6 the use of *S. thermophilus* instead of *L. acidophilus*. From the graphs, it appears that neither *L. bulgaricus* nor *S. thermophilus* (the dotted lines) are inhibited by the growth of *C. albicans*. It appears from Fig. 5 that there is a slight inhibition of *C. albicans* when grown together with *L. bulgaricus*. From Fig. 6 it appears that *S. thermophilus* does not exert any inhibitory effect against *C. albicans*.

The above tests indicate that the chosen strain of *L. acidophilus* shows the most efficient inhibition on *C. albicans* compared with *L. bulgaricus* whereas no effect was shown from *S. thermophilus*.

Fig. 7 shows that, when a broth is acidified (to a pH of 4.18 by means of the selected strain of *L. acidophilus*) and physically sterilized as described above, the *C. albicans* culture (indicated by the "white" circle) cannot tolerate the concentration of the antimicrobial metabolites, i.e. the cell counts drop during the first 24 hours period. In comparison, the growth of *C. albicans* in normal broth is indicated by the filled-in circle.

A chemical acidification of MRS-broth with lactic and phosphoric acid to a pH 4-4.2 has no influence, or only very little influence, on the growth of *C. albicans*. Thus, it is demonstrated that it is the production of antimicrobial agent by certain lactic acid bacteria, principally *L. acidophilus*, which is responsible for the inhibition in the growth of yeast cells.

EXAMPLE 6

Inhibition of β-streptococci (Streptococcus Group B*) in vitro*

In the field of human pathology, β-*Streptococci* are recognized as important infectious agents especially in the urogenital area, and a significant correlation has been demonstrated between the presence of β-*streptococci* and the diagnosis of vaginitis (cf. Ugeskrift for Læger *141*/15, 1979, pp. 992-994; *Scand. J. Infect. Dis. 11*, 1979, pp. 199-202; *Scand. J. Infect. Dis. 12*, 1980, pp. 101-104). To demonstrate the inhibitory effect of *L. acidophilus* on the growth of these potential pathogens *in vitro* experiments were performed essentially as described in Example 5 using laboratory reference strains from Statens Veterinære Seruminstitut, Ringsted, Denmark (Types IA, IB, II and III). It appeared from these tests that the growth of β-*streptococci* is inhibited when the streptococci are grown together with *L. acidophilus* (partly a question of substrate competition). The growth is also largely inhibited in a substrate with a pH below about 5.00 (the optimal pH of β-*streptococci* is about 7.4). It was also demonstrated that the growth is inhibited more than 2 logs in a neutralized MRS-broth in which *L. acidophilus* had been grown, compared to an MRS-substrate to which the equivalent amount of sodium lactate had been added.

Thus, it was demonstrated that *L. acidophilus* production of antimicrobial agents combined with the decrease in pH is responsible for the inhibition of the growth of β-*streptococci*.

EXAMPLE 7

Inhibition of vulvo-vaginal infections in vivo

Preliminary clinical studies of the inhibitory effect of the capsules of the invention on vulvo-vaginal infections were carried out at the dermatological clinics of several hospitals.

I. The study was performed at the dermatological clinic of Odense Sygehus, Denmark, involving 8 women of ages between 18 and 30 years (23 years on average) suffering from vulvo-vaginal infections, who had been referred to the clinic for treatment. The test comprised administering one capsule twice daily for 7 days so that four of the patients received the capsule of the invention and four of the patients received a placebo capsule used as control. After 1 week, a subjective as well as objective evaluation of the condition of the patients was carried out, comparing it to their condition before treatment. The criteria of evaluation were: recovery (scored as 1), improvement (2), no change (3), and deterioration (4).

By a t-statistical analysis of the means of two samples, the following results were obtained.

Table 4

Evaluation	Mean		Standard deviation		t-test prob. t>
	Capsule of the invention	Placebo	Capsule of the invention	Placebo	
Subjective	1.75	3	0.5	0.0	0.0012
Objective	2.25	3	0.5	0.816	0.084

The subjective evaluation of the effect of the capsule of the invention compared to the placebo capsule showed a statistical significance of 99.88%, whereas the objective evaluation is almost significant at a level of 91.6%.

II. The study was carried out at the dermatological clinic of Rigshospitalet, Copenhagen, Denmark, involving 10 women of ages between 21 and 43 years (average age: 30 years) who had suffered from chronic or recurrent vaginitis for 1/2-8 years (3 years on average). The symptoms were constant in 2 patients, recurred once a month in 6 patients and ocurred 4-10 times a year in 2 patients.

The capsule according to the invention was administered once a day for 2-6 months (4 months on average). During the period of treatment, *C. albicans* infections recurred in 6 of the patients, and the presence of *Gardnerella vaginalis* was demonstrated in 3 cases. 2 Patients received antibiotics and 3 patients received antimycotics for brief periods during the period of treatment with the capsule of the invention.

After the test period one patient reported recovery and 3 patients reported their condition improved (abating symptoms at greater intervals). In 4 patients no change had occurred after 2 months' treatment, and 2 patients reported a deterioration of their condition (increased irritation of the vaginal mucosa) after 3 months' treatment.

In the summary of the test, it was concluded that the capsule of the invention is also useful in the treatment of women suffering from severe, chronic or recurrent vaginitis.

III. In a subjective study of the inhibition of vulvo-vaginal infections, a group of 27 women of ages between 17 and 38 years who suffered from recurrent vulvo-vaginal infections which had repeatedly been treated with the usual vaginal compositions, were treated by administering one capsule of the invention a day for 6 subsequent days.

Results (subjective evaluation):

5 patients reported that the capsules had no effect,
12 patients reported that the capsules had had a satisfactory effect,
8 patients reported that the capsules had had an excellent effect, and
2 patients did not report.

CLAIMS

1. A soluble capsule for controlling vulvo-vaginal infections which contains a stable, dried, viable concentrate of lactic acid bacteria dispersed in a pharmaceutically acceptable fluid carrier which has a viscosity of at least 2000 cps.

2. A capsule according to claim 1 in which the fluid carrier has a viscosity of at least 3000 cps, in particular at least 4000 cps.

3. A capsule according to claim 1 which is a gelatin capsule, in particular a soft gelatin capsule.

4. A capsule according to any of claims 1-3 in which the count of viable lactic acid bacteria in the capsule is at least 1×10^6, preferably at least 1×10^7.

5. A capsule according to claim 4 in which the count of lactic acid bacteria in the capsule is at least 1×10^8, preferably in the range between about 1×10^9 and 1×10^{11}, such as about 1×10^{10}.

6. A capsule according to any of claims 1-5 in which the concentrate is a freeze-dried concentrate.

7. A capsule according to claim 6 in which the freeze-dried concentrate has a water activity in the range of 0.00-0.2, preferably 0.00-0.1.

8. A capsule according to any of claims 1-7 in which the fluid carrier is a substantially anhydrous paste.

9. A capsule according to claim 8 in which the paste has a water activity in the range of 0.00-0.2, preferably 0.00-0.1.

10. A capsule according to any of claims 7-9 in which the paste comprises a substantially non-hygroscopic oil.

11. A capsule according to claim 10 in which the oil contains a viscosity-increasing agent.

12. A capsule according to claim 11 in which the viscosity-increasing agent is a substance with a water activity of not more than 0.2, preferably not more than 0.1.

13. A capsule according to claim 11 or 12 in which the viscosity-increasing ingredient is selected from solid or semi-solid hydrocarbons capable of forming a homogeneous system with the oil, such as vaseline or polyethylene, particulate inorganic substances such as fumed silica, talc, zeolite or bentonite, or carbohydrates or carbohydrate derivatives, preferably high molecular weight carbohydrates such as starch and starch derivatives, or a mixture thereof.

14. A capsule according to claim 13 in which the viscosity-increasing agent is a starch.

15. A capsule according to claim 14 in which the starch is corn starch.

16. A capsule according to claim 14 or 15 in which the starch is admixed with vaseline.

17. A capsule according to claim 16 in which the amount of vaseline is about 10-50% by weight of the starch.

18. A capsule according to any of claims 10-17 in which the oil is a mineral or vegetable oil such as paraffin or sunflower oil.

19. A capsule according to any of claims 15-18 in which the weight ratio between the oil and the starch or starch/vaseline mixture is in the range from about 2:5 to about 5:2, preferably about 1:1.

20. A capsule according to any of claims 1-19 in which the lactic acid bacteria are of the genus *Streptococcus* or *Lactobacillus*.

21. A capsule according to claim 20 wherein the species of *Lactobacillus* are *L. acidophilus, L. bulgaricus, L. lactis, L. helveticus, L. bifidus, L. casei, L. plantarum, L. delbrueckii, L. thermophilus, L. fermentum* or a mixture thereof.

22. A capsule according to claim 21 wherein the strain of *L. acidophilus* is *L. acidophilus* NRRL No. B-15260.

23. A capsule according to claim 20 wherein the species of *Streptococcus* are *S. lactis, S. cremoris, S. diacetylactis, S. thermophilus, S. faecium* or a mixture thereof.

24. A method of producing a soluble capsule containing a stable dried viable concentrate of lactic acid bacteria, comprising homogeneously dispersing a dried, viable, stable culture of lactic acid bacteria in a substantially anhydrous fluid carrier, filling the resulting dispersion into soft gelatin capsules and drying the capsules after sealing.

25. A method according to claim 24 in which the fluid carrier has a viscosity of at least 2000 cps, preferably at least 3000 cps, in particular at least 4000 cps, at 20°C.

26. A method according to claim 24 or 25 in which the fluid carrier comprises a pharmaceutially acceptable, substantially anhydrous, non-hygroscopic oil.

27. A method according to claim 26 in which the fluid carrier additionally comprises a viscosity-increasing agent.

28. A method according to claim 27 in which the viscosity-increasing agent is a substance with a water activity of not more than 0.2, preferably not more than 0.1.

29. A method according to claim 28 in which the viscosity-increasing agent is selected from solid or semi-solid hydrocarbons capable of forming a homogeneous system with the oil, such as vaseline or polyethylene, particulate inorganic substances such as fumed silica, talc,

zeolite or bentonite or carbohydrates or carbohydrate derivatives, preferably high molecular weight carbohydrates such as starch and starch derivatives.

30. A method according to claim 29 in which the viscosity-increasing agent is a starch.

31. A method according to claim 30 in which the starch is corn starch.

32. A method according to claim 30 or 31 in which the starch is admixed with vaseline.

33. A method according to claim 32 in which the amount of vaseline is about 10-50% by weight of the starch.

34. A method according to any of claims 26-33 in which the oil is a mineral or vegetable oil such as paraffin or sunflower oil.

35. A method as according to any of claims 30-34 in which the weight ratio between the oil and the starch or starch/vaseline mixture is in the range from about 2:5 to about 5:2, preferably about 1:1.

36. A method according to any of claims 24-35 in which the bacterial concentrate is dispersed in the fluid carrier in an amount which is sufficient to secure a cell count in the final capsules of at least 1×10^6, after which the viscosity-increasing agent is added.

37. A method according to any of claims 24-36 in which the concentrate is a freeze-dried concentrate.

38. A method according to claim 32 in which the freeze-dried concentrate has a water activity in the range of 0.00-0.2, preferably 0.00-0.1.

39. A method of controlling vulvo-vaginal infections which comprises intravaginally administering a capsule containing a stable, dried,

viable concentrate of lactic acid bacteria dispersed in a pharmaceutically acceptable fluid carrier.

40. A method according to claim 39 in which the count of viable lactic acid bacteria in the capsule is at least 1×10^6, preferably at least 1×10^7.

Fig. 1.

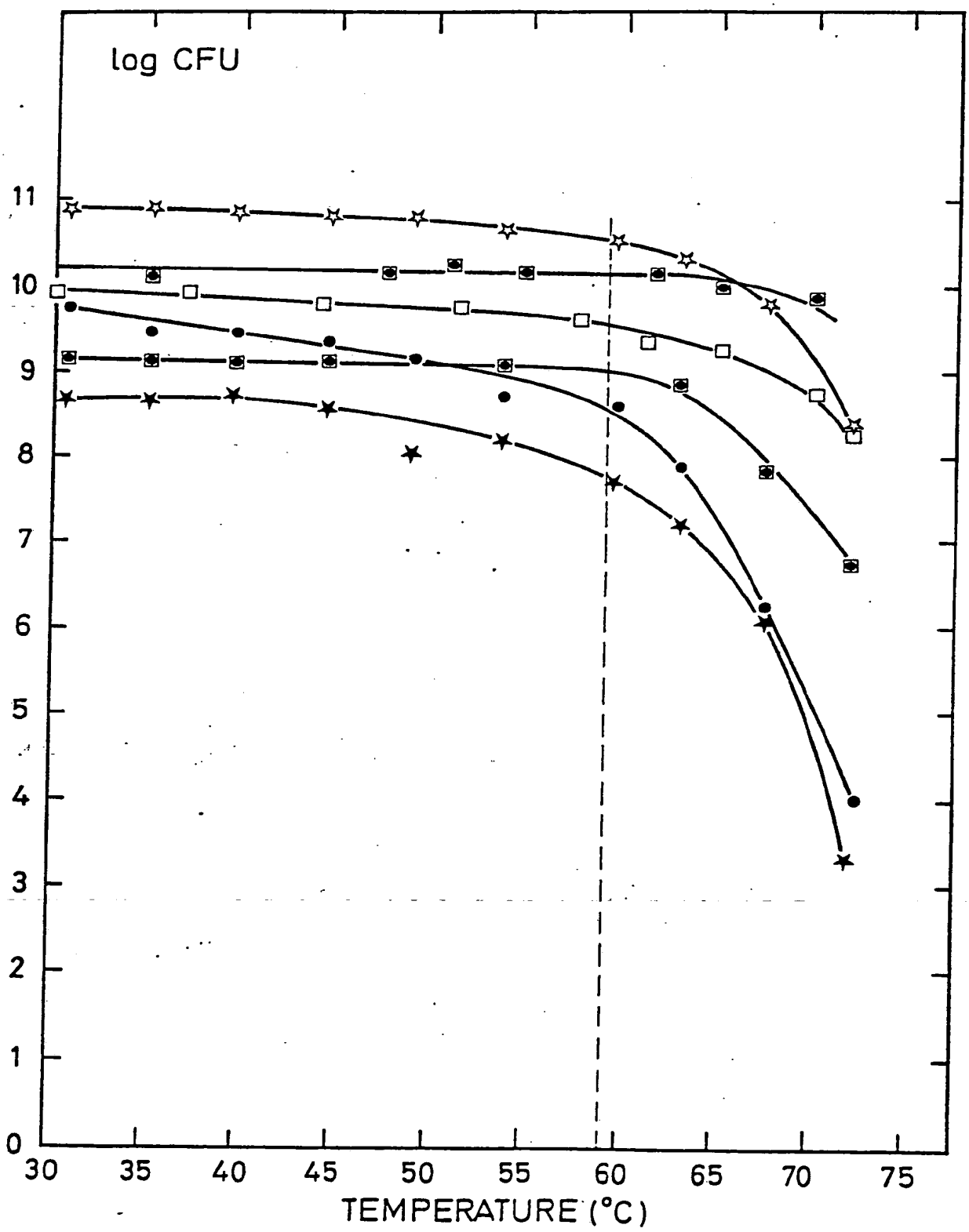


Fig. 2.

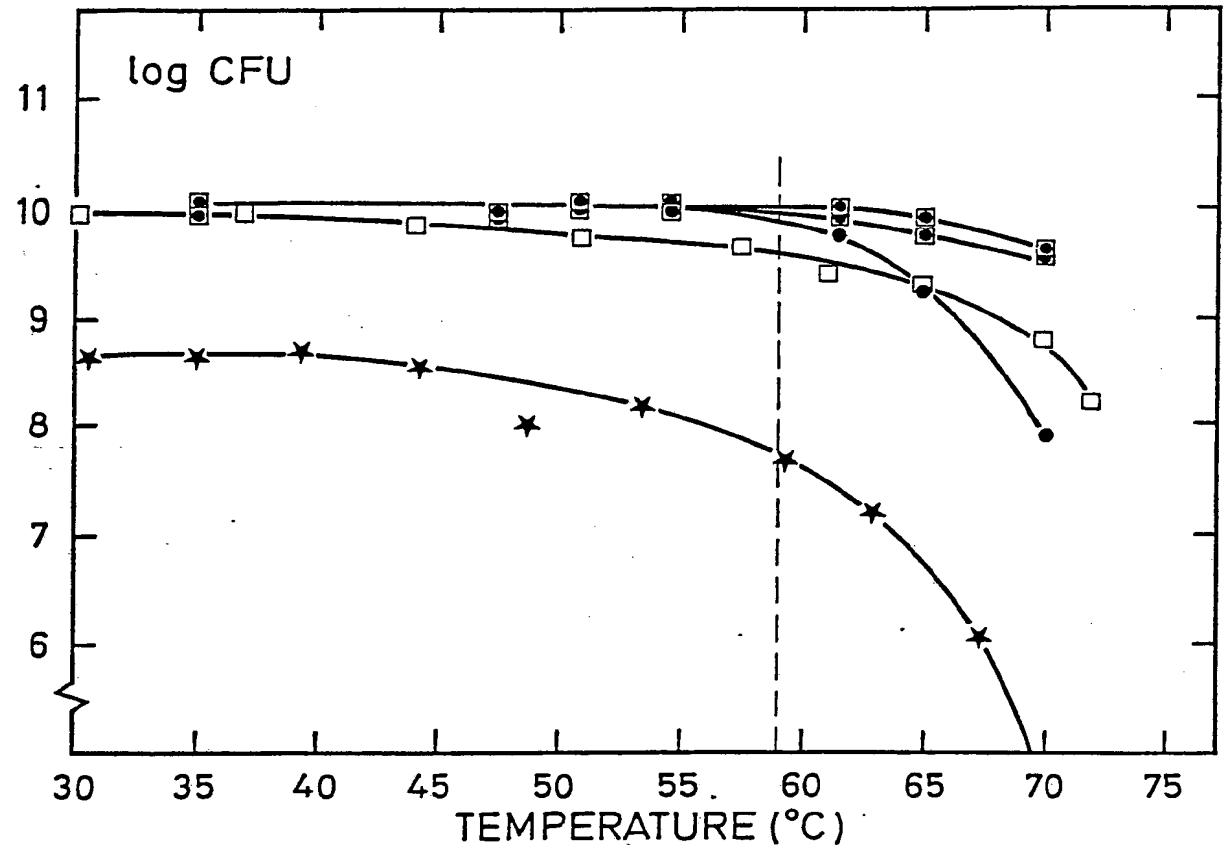


Fig. 3.

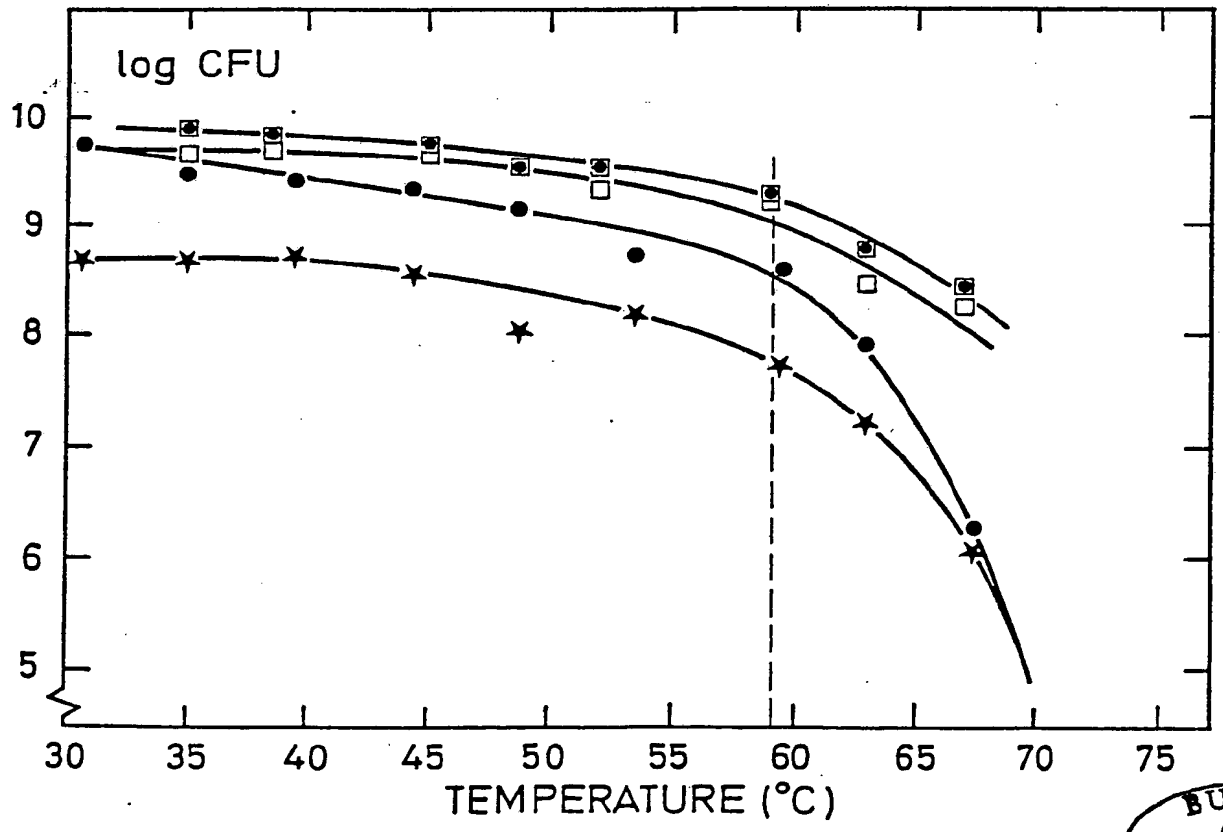


Fig. 4a.

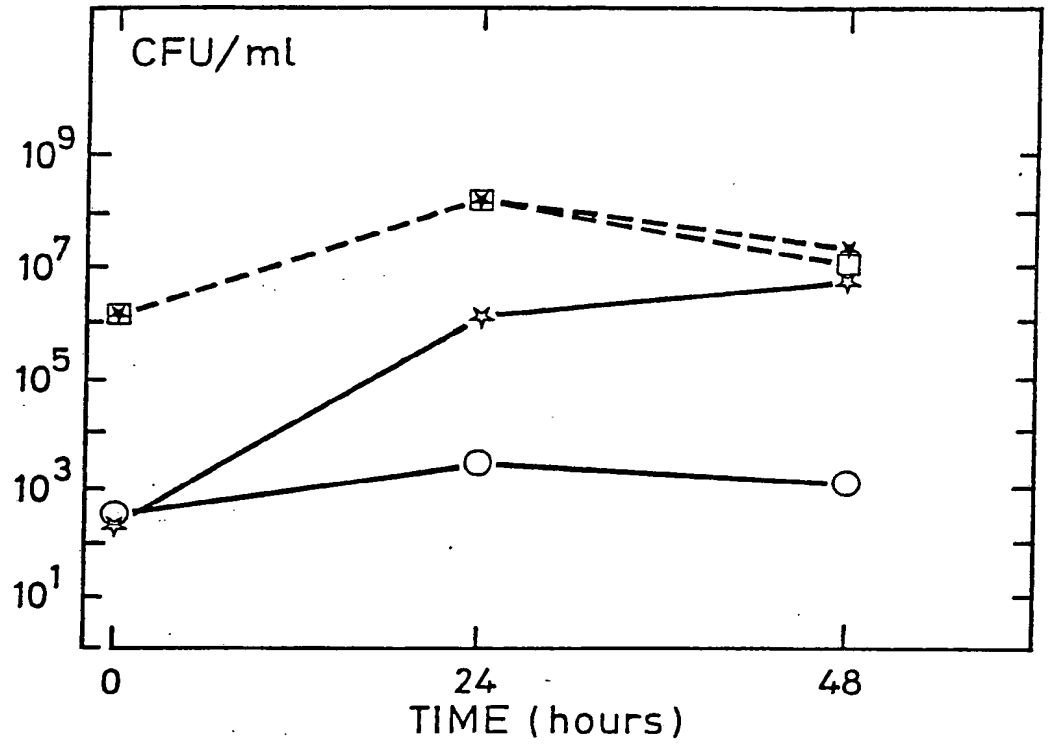
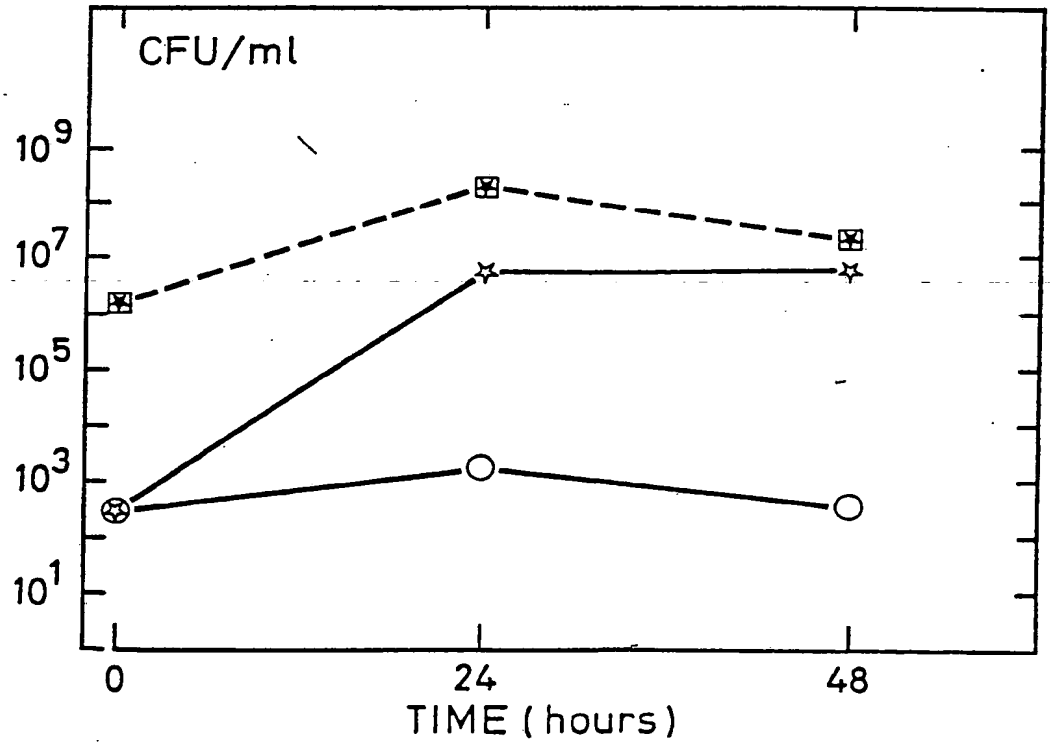


Fig. 4b.



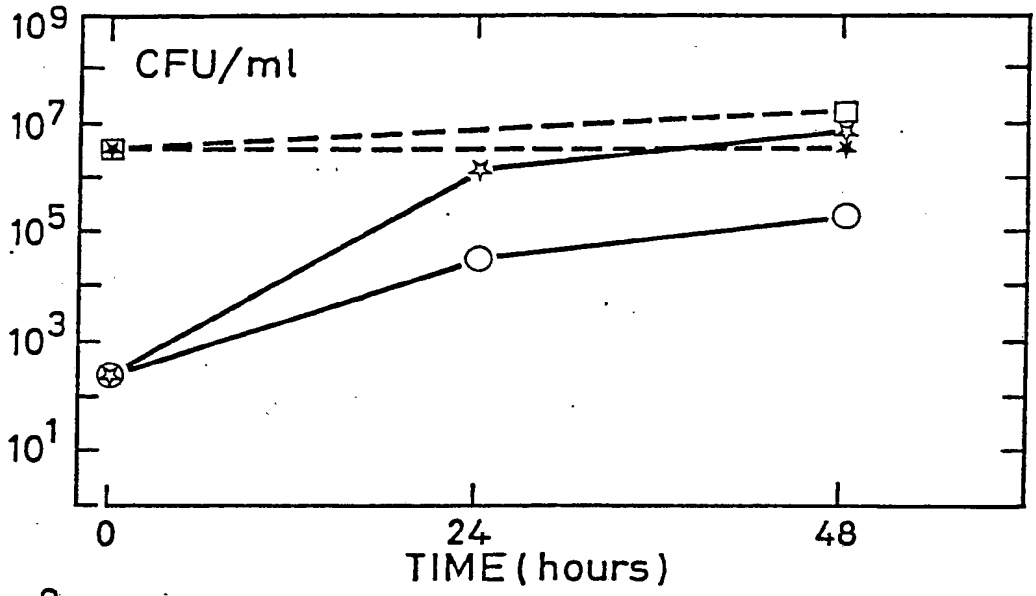


Fig. 5.

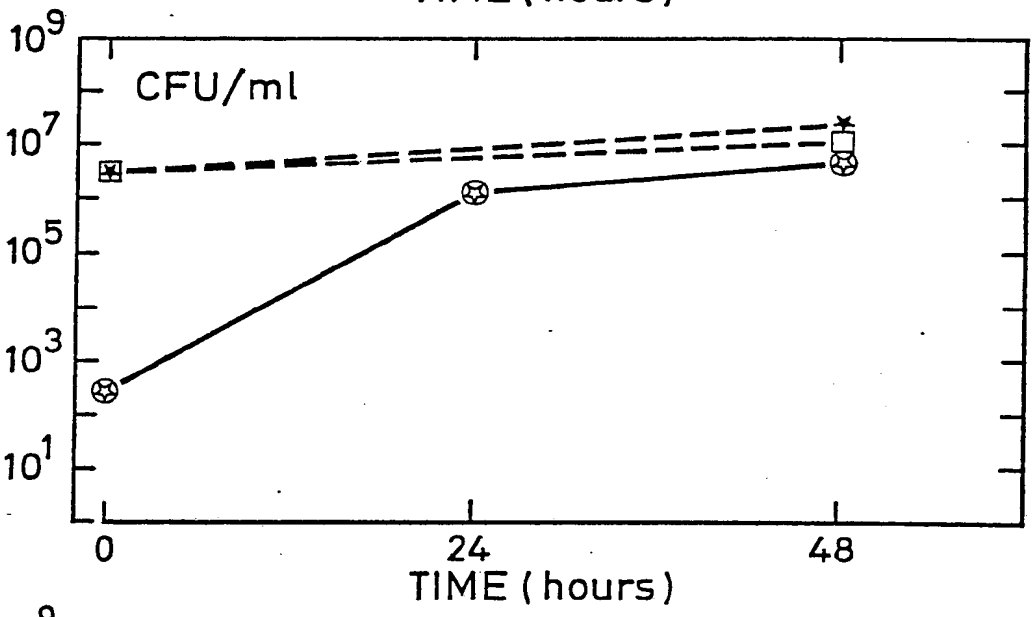


Fig. 6.

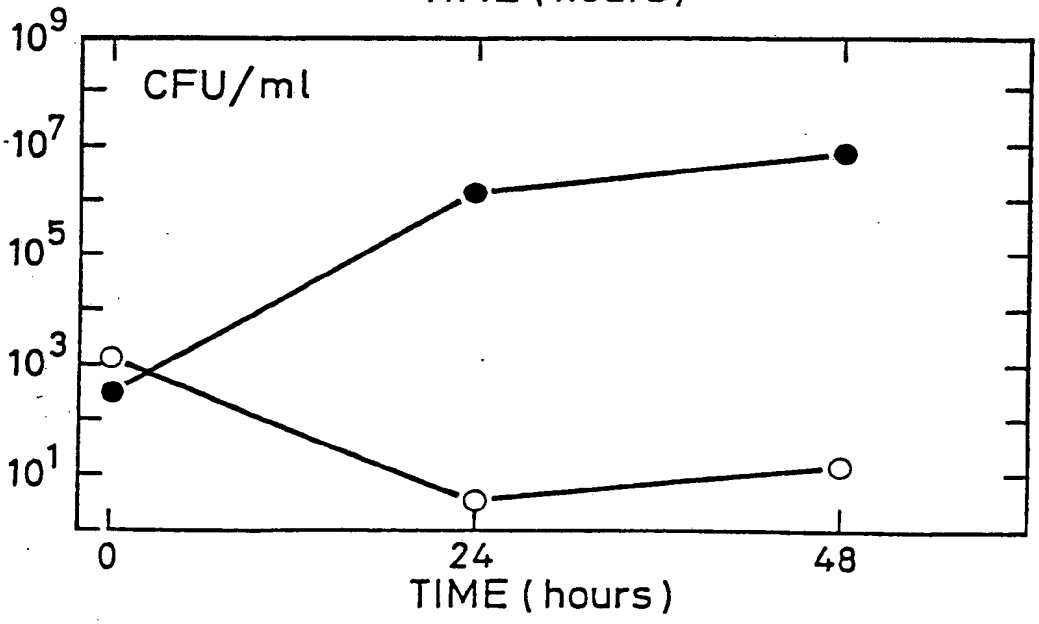


Fig. 7.

INTERNATIONAL SEARCH REPORT

International Application No PCT/DK84/00042

I CLASSIFICATION F SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC 3

A 61 K 9/48

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
IPC 3	A 61 K 9/48, 9/06, 9/08, 9/14, 9/12, 9/02, 9/10, 9/00, 9/16; A 61 K 35/66, 35/74; C 12 K 3/00
National Cl	30h:9/03; 30h:9/04; 30h:9/06 .../...

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [5]

SE, NO, DK, FI classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category [6]	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X	DK, A, 127 268 (THE GREEN CROSS CORPORATION) 15 October 1973	1-40
X	DE, A, 2 738 652 (SEIKEN KAI FOUNDATIONAL JURIDICAL PERSON) 15 March 1979	1-40
A	DE, A, 2 448 648 (DSO, PHARMACHIM) 24 April 1975	1-40
A	DE, A, 605 803 (IWAN ARBATSKY IN BORGSDORF b BERLIN) 1 November 1934	1-40

[6] Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [3]	Date of Mailing of this International Search Report [3]
1984-07-10	1984-07-20
International Searching Authority [1]	Signature of Authorized Officer [20]
Swedish Patent Office	Terttu Gierer

L.E.

Form PCT/ISA/210 (second sheet) (October 1981)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

II Fields searched (cont).

US Cl 424:14

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____, because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers _____, because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the International application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the International application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.